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Research Note

Use of Pulsed-Field Gel Electrophoresis To Monitor a Five-Strain Mixture of *Listeria monocytogenes* in Frankfurter Packages†

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ABSTRACT

In a previous study, the viability of a five-strain mixture of *Listeria monocytogenes* (including Scott A [serotype 4b, clinical isolate], 101M [serotype 4b, beef-pork sausage isolate], F6854 [serotype 1/2a, turkey frankfurter isolate], H7776 [serotype 4b, frankfurter isolate], and MFS-2 [serotype 1/2a, pork plant isolate]) was monitored during refrigerated storage of frankfurters prepared with and without 3.0% added potassium lactate. Throughout a 90-day period of storage at 4°C, the initial inoculum level of 20 CFU per package remained relatively constant in packages containing frankfurters prepared with potassium lactate, but pathogen counts increased to 4.6 $\log_{10}$ CFU in packages containing frankfurters prepared without added potassium lactate. To determine which of the five strains persisted under these conditions, randomly selected colonies obtained after 28 and 90 days of refrigerated storage of frankfurters were analyzed by pulsed-field gel electrophoresis (PFGE) with the restriction enzyme *Sma*I to generate distinct banding patterns for each of the five strains. Then, with the use of PFGE as a tool for identification, the percentages of the strains on days 28 and 90 of the growth study were compared. In the absence of any added potassium lactate in the product, 43% of the 58 isolates recovered on day 28 were identified as strain Scott A, 12% were identified as strain 101M, 22% were identified as strain F6854, 10% were identified as strain H7776, and 12% were identified as strain MFS-2. However, by day 90, an appreciable number (83%) of the 60 isolates analyzed were identified as strain MFS-2. In packages containing frankfurters formulated with 3.0% potassium lactate, all five strains were present at frequencies of 5 to 36% among the 19 isolates tested on day 28; however, by day 90, strain MFS-2 made up the statistical majority (63%) of the 27 isolates tested. The results of this study indicate that strain MFS-2, a serotype 1/2a isolate recovered from a pork processing plant, was more persistent than strains Scott A, 101M, F6854, or H7776 during the extended refrigerated storage of frankfurters.

Despite the availability of much information about the survival and growth of *Listeria monocytogenes* in frankfurters, few studies have assessed how individual strains in a mixture of *L. monocytogenes* strains may fare in packages of frankfurters and/or whether certain strains may exhibit selective advantages over other strains. The ability to precisely track individual strains in such a complex environment would be quite useful given that some strains display varying responses to environmental stresses *(2, 7, 8, 10)* and that some strains are more virulent than others *(1, 3, 5, 6, 11)*. In a previous study, we reported on the fate of a five-strain mixture of *L. monocytogenes* during 90 days of refrigerated storage in vacuum-sealed packages of frankfurters formulated with and without 3.0% added potassium lactate *(9)*. The purpose of the present study was to use pulsed-field gel electrophoresis (PFGE) to determine the percentages and persistence levels of the five *L. monocytogenes* strains in the previous experiment.

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† Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

MATERIALS AND METHODS

Inoculation and vacuum packaging of frankfurters. The inoculation of vacuum-packaged frankfurters with stationary-phase cells of *L. monocytogenes* has previously been described *(9)*. Briefly, about 20 CFU of *L. monocytogenes,* or about 4 CFU of each of five *L. monocytogenes* strains (including Scott A [serotype 4b, clinical isolate], 101M [serotype 4b, beef-pork sausage isolate], F6854 [serotype 1/2a, turkey frankfurter isolate], H7776 [serotype 4b, frankfurter isolate], and MFS-2 [serotype 1/2a, pork plant isolate]) (Table 1) per package, were used to inoculate packages of commercially prepared frankfurters, which were then incubated at 4°C for 90 days. Two different beef-pork frankfurter formulations were tested. One formulation contained potassium lactate added to the batter at a final concentration of ca. 3.0%, and the other formulation did not contain any added potassium lactate.

Microbiological methods. The viability of *L. monocytogenes* over 90 days of incubation at 4°C was established by direct plating on modified Oxford (MOX) agar *(9)*. For each formulation, up to 60 *L. monocytogenes* colonies were randomly isolated from the day 28 and day 90 MOX plates and stored at −80°C in brain heart infusion broth (Difco Laboratories, Sparks, Md.) plus 10% glycerol (Sigma Chemical Co., St. Louis, Mo.). Two trials

TABLE 1. *Strains of* L. monocytogenes *used to inoculate packages of commercially prepared frankfurters*

Strain	Serotype	Source[a]
Scott A	4b	Clinical isolate
101M	4b	Beef-pork sausage
F6854	1/2a	Turkey frankfurter
H7776	4b	Frankfurter
MFS-2	1/2a	Pork processing plant

[a] Porto et al. *(9).*

were carried out, and three packages of frankfurters were tested on each sampling day.

Pulsed-field gel electrophoresis and statistical methods. The protocol of Graves and Swaminathan *(4)* was used for PFGE. Gels were stained with a 5% ethidium bromide solution and photographed with Multi-Analyzer gel documentation software (Bio-Rad, Hercules, Calif.). For statistical analysis of the results, a chi-square test was used to determine whether the observed recovery rates of the five strains were significantly different from equal recovery rates for the five strains. In addition, the recovery rates of strain MFS-2 for day 90 in both frankfurter formulations were compared by a chi-square test.

RESULTS AND DISCUSSION

Each of the five strains of *L. monocytogenes* was characterized by PFGE with restriction endonucleases *Asc*I, *Apa*I, and *Sma*I to evaluate which enzyme would provide the most distinguishing banding patterns (data not shown). The restriction enzyme *Sma*I generated the most readily discernible fragments for each of the five strains of *L. monocytogenes* used in this study (Fig. 1).

In a previous study *(9),* the viability of the five-strain mixture of *L. monocytogenes* in vacuum-sealed packages of frankfurters formulated with and without 3.0% added potassium lactate was subsequently monitored by enumeration on MOX agar plates. Briefly, no growth occurred in packages containing frankfurters formulated with 3.0% added potassium lactate after 90 days of incubation, most likely because of the antilisterial effects of potassium lactate. In packages with no added potassium lactate, a 4.6-$\log_{10}$ increase in *L. monocytogenes* cell numbers was observed after 90 days of incubation. Similarly, the growth of indigenous lactic acid bacteria (LAB) was determined by plating on Rogosa SL agar *(9).* In the absence of any added potassium lactate, the LAB count increased from an initial level of 2.6 $\log_{10}$ CFU per package to 7.6 $\log_{10}$ CFU per package after 90 days, whereas the LAB count increased from an initial level of 0.7 $\log_{10}$ CFU per package to 10.6 $\log_{10}$ CFU per package after 90 days of incubation in the presence of 3.0% added potassium lactate *(9).*

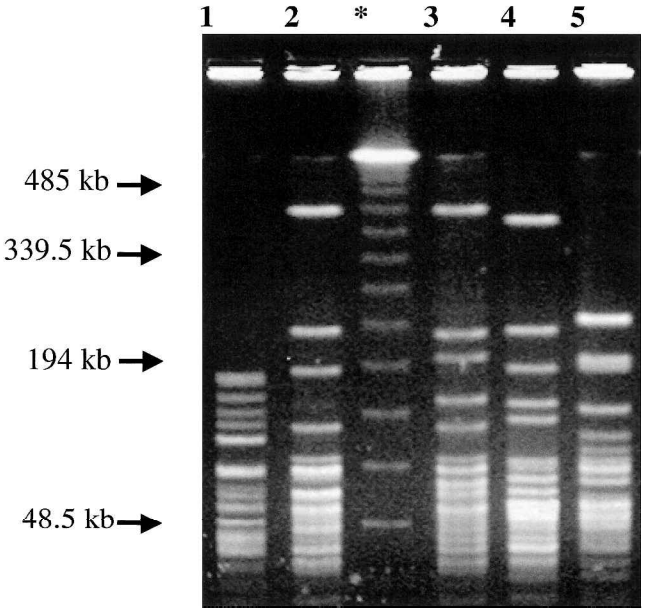

FIGURE 1. Sma*I-generated PFGE restriction patterns for the five* L. monocytogenes *strains used in this study. Electrophoresis conditions were 14°C and 200 V, with pulses ramping from 4 to 40 s over 22 h. Arrows denote the approximate DNA sizes as determined from the lambda DNA sizing ladder (BioRad). Lanes: 1, strain F6854; 2, strain 101M; *, ladder; 3, strain H7776; 4, strain Scott A; 5, strain MFS-2.*

For the packages containing frankfurters prepared without added potassium lactate, colonies were recovered from the MOX plates used to enumerate the pathogen after 28 (58 isolates) and 90 (60 isolates) days of incubation at 4°C. PFGE analysis of the 58 isolates recovered on day 28 demonstrated that each strain accounted for 10 to 43% of the total number of isolates recovered (Table 2). A statistical analysis of the recovery rates suggested that strain Scott A was recovered at a significantly ($P = 0.0002$) higher rate than the other four strains were. However, by day 90, analysis of the 60 isolates recovered revealed that strain MFS-2 accounted for a statistically significant ($P < 0.0001$) majority of the isolates (83%; 50 of 60 isolates) and that the remaining four strains each accounted for 0 to 7% of the recovered *L. monocytogenes* population. For packages containing frankfurters formulated with 3.0% added potas-

TABLE 2. *Percentages of* L. monocytogenes *strains Scott A, 101M, H7776, F6854, and MFS-2 in vacuum-sealed packages of frankfurters containing 0% and 3% added potassium lactate after 28 and 90 days of storage at 4°C*

Formulation	Day (*n*)[a]	% (no.) of isolates recovered for strain				
		Scott A	101M	H7776	F6854	MFS-2
No added potassium lactate	28 (58)	43 (25)	12 (7)	10 (6)	22 (13)	12 (7)
	90 (60)	0 (0)	5 (3)	5 (3)	7 (4)	83 (50)
3% added potassium lactate	28 (19)	37 (7)	21 (4)	26 (5)	5 (1)	11 (2)
	90 (27)	7 (2)	4 (1)	7 (2)	19 (5)	63 (17)

[a] *n,* total number of isolates recovered.

sium lactate, colonies were also recovered from the MOX plates used to enumerate *L. monocytogenes* after 28 (19 isolates) and 90 (27 isolates) days of incubation at 4°C. PFGE analysis of the 19 isolates recovered after 28 days revealed that all five strains were isolated, with each strain accounting for 5 to 37% of the isolates recovered (Table 2). There was no statistical evidence that any single strain was recovered at a significantly ($P = 0.1991$) higher or lower frequency than any other strain on day 28. After 90 days of storage at 4°C, 27 isolates were recovered, and strain MFS-2 predominated, accounting for a significant ($P < 0.0001$) majority of the recovered *L. monocytogenes* population (63%, 17 of 27 isolates). The remaining isolates of *L. monocytogenes* recovered on day 90 from packages containing frankfurters prepared with 3% added potassium lactate were F6854 (19%), H7776 (7%), 101M (4%), and Scott A (7%).

The results obtained indicate that strain MFS-2 was able to "outcompete" strains F6854, H7776, 101M, and Scott A in the specific frankfurter formulations and under the conditions used in this study. Strain MFS-2 was recovered at a significantly ($P = 0.0367$) higher frequency from packages of frankfurters prepared without added potassium lactate than from packages of frankfurters prepared with 3% added potassium lactate. This result could be attributable in part to the occurrence of more total growth of the pathogen in packages containing frankfurters without added potassium lactate, providing more opportunity for the growth of strain MFS-2. However, other factors, such as the presence, levels, and types of LAB, the product formulation, the presence of organic acids, and strain MFS-2's genetic makeup per se, may also have contributed to the predominance of this strain.

Another characteristic that may vary among the different isolates of *L. monocytogenes* is the level of resistance to bacteriocins. In a study by Buncic et al. *(2),* a panel of serotype 1/2a strains were more resistant to two antilisterial bacteriocins than serotype 4b strains were in laboratory media at 4°C. These authors concluded that serotype 1/2a strains may have a competitive advantage over serotype 4b strains in cold-stored meats, wherein LAB comprise the majority of the spoilage flora and are known to produce antilisterial bacteriocins *(2).* Similarly, in the present study, the presence of high levels of indigenous LAB in the frankfurter packages may have generated bacteriocins or other antimicrobial metabolites that contributed to the prevalence of strain MFS-2, a serotype 1/2a strain. However, the production of bacteriocins and/or antimicrobial metabolites by the indigenous LAB was not investigated in this study. The other serotype 1/2a strain used in this study, strain F6854, did not predominate in the packages of frankfurters prepared without added potassium lactate. However, after 90 days of incubation in the packages of frankfurters prepared with added potassium lactate, strain F6854 was recovered at a frequency of 19%, a recovery rate higher than those observed for the three serotype 4b strains tested. Further research is needed to determine whether serotype 1/2a strains such as MFS-2 and F6854 are indeed more tolerant of LAB and/or bacteriocins and antimicrobial metabolites produced by LAB in vacuum-packaged frankfurters than are serotype 4b strains.

The finding that strain MFS-2, which was initially isolated from a pork processing plant, predominated in this study suggests that this strain may be more adapted to processed meat products than are the other strains studied. Furthermore, strain MFS-2 was previously obtained from the same pork processing plant that produced the frankfurters used in the present study, albeit nearly 2 years prior to the production of the batch of frankfurters used herein. Perhaps more significant, strain MFS-2 survived better in the vacuum-sealed packages of frankfurters than did strains F6854 and H7776, which were originally isolated from frankfurters. It is possible that a given formulation for frankfurters may select for a particular strain of *L. monocytogenes.* Although such adaptation theories have scientific merit, more studies are necessary to confirm these theories.

In summary, the results of the present study indicate that the source of an *L. monocytogenes* isolate may not be indicative of its particular adaptive ability, since in the present study, an environmental isolate was able to grow better in vacuum-sealed packages of frankfurters than were strains isolated from frankfurters, sausage, or a human patient. Further studies are warranted to determine how *L. monocytogenes* strains persist and predominate in food environments, particularly in ready-to-eat foods, for which contamination by *L. monocytogenes* is a major public health risk. To our knowledge, this is the only published study that has definitively monitored the progress of several *L. monocytogenes* strains during the refrigerated storage of foods. The PFGE technique was essential for accomplishing this task, and it will be useful for further studies on strain competition occurring in a food environment.

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